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RESPONSE OF NORTHERN CORN ROOTWORM, *Diabrotica barberi* SMITH AND LAWRENCE,[1] TO STEREOISOMERS OF 8-METHYL-2-DECYL PROPANOATE[2]

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Abstract—The four stereoisomers of 8-methyl-2-decyl propanoate were tested in South Dakota for attractiveness to the northern corn rootworm, *Diabrotica barberi* Smith and Lawrence (NCR). Only the 2*R*,8*R* configuration was attractive to the NCR. Inhibition of the NCR response to 2*R*,8*R* occurred when either the 2*S*,8*R* or 2*S*,8*S* isomers were components of the pheromone source. The 2*R*,8*S* configuration elicited no behavioral activity in the NCR.

Key Words—Chrysomelidae, *Diabrotica barberi*, northern corn rootworm, sex pheromone, stereospecificity, inhibition, enantiomer.

INTRODUCTION

The northern corn rootworm, *Diabrotica barberi* Smith and Lawrence (NCR), is a common pest of corn east of the Rocky Mountains in both the U.S. and Canada. The NCR is often found cohabiting with the western corn rootworm, *D. virgifera virgifera* Le Conte (WCR), with which it shares a similar life history (Branson and Krysan, 1981).

[1]Coleoptera: Chrysomelidae.
[2]Mention of a commercial or proprietary product does not constitute an endorsement by the USDA.
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Until recently, widespread uncertainty existed as to whether or not the NCR and WCR were distinct species (Chiang, 1973). This confusion stemmed from frequent field observations of apparent interspecific matings, observations and collections of putative hybrids, and the reported production of hybrids in the laboratory (Hintz and George, 1979). Notwithstanding the latter, Krysan and Guss (1978) showed that, although hybridization between the NCR and WCR is possible under laboratory conditions, reproductive barriers between the two species exist to the extent that hybridization under natural conditions is unlikely.

It is well documented that females of the NCR and WCR are cross-attractive to males of either species. Guss (1976) showed that extracts from virgin WCR females attracted males of both the WCR and NCR, although a temporal difference in response was demonstrated, i.e., WCR males responded during daylight hours while NCR males responded at night. These observations were confirmed by Bartelt and Chiang (1977), who further showed that virgin females of both species attracted males of either species to baited traps.

The sex pheromone of the WCR was identified as 8-methyl-2-decyl propanoate (Guss et al., 1982), and subsequent studies indicated that the configuration of the naturally produced pheromone was 2*R*,8*R* (Guss et al., 1983). At low doses, racemic 8-methyl-2-decyl propanoate was attractive to males of the NCR, but at loadings of 10 μg or more, dispensed from rubber septa, the response of NCR males to the synthetic racemate was extinguished (Guss et al., 1982). This result suggested, among other possibilities, that one or more of the stereoisomers in racemic 8-methyl-2-decyl propanoate was inhibiting the response by male NCR to the active component(s).

We have recently reported on the response of the WCR and two other *Diabrotica* to the individual stereoisomers of 8-methyl-2-decyl propanoate (Guss et al., 1983. This report deals with the response of the NCR to those same isomers.

METHODS AND MATERIALS

The stereoisomers used in this study are the same preparations used earlier (Guss et al., 1983). Syntheses of these components were accomplished by a convergent approach in which two fragments, each containing one asymmetric center, were joined to complete the required sequence. The configurational purity of each fragment, which assured the configurational purity of the final product, was determined absolutely by GLC and/or HPLC using diastereomeric derivatives (Sonnet and Heath, 1982; Carney et al., unpublished). Isomeric purity of the target isomers was: 2*R*,8*R* (97.8%); 2*R*,8*S* (98.3%); 2*S*,8*R* (97.4%); and 2*S*,8*S* (98.8%).

The individual isomers, or specific mixtures, were diluted to appropriate concentrations in hexane and dispensed into the "cup" portion of rubber septa (A.H. Thomas No. 8753-D22) in 50 μl quantities to produce the pheromone sources. The total amount of pheromone per source was limited to 1μg when testing individual isomers or 3.5 μg in mixtures, to obviate as much as possible effects attributable to the small amounts of nontarget isomers in these preparations.

Pheromone traps were constructed from plastic-coated milk carton blanks, and, when deployed, were the shape of a vertically oriented triangular prism 9 × 20 cm on each face. The traps, coated on the outside with Tangle-Trap, were placed on wooden stakes (1 m) constructed from lathing (1.0 × 3.5 cm) and equipped with a 20-cm-long crossbar 20 cm from the top. To ensure that the sticky surfaces of the traps did not lose their trapping efficiency because of accumulation of dirt and debris, fresh traps were placed on the stakes daily in all experiments with one exception. In the third test (Table 3) traps were deployed for five days and changed four times (24 hr, 24 hr, 24 hr, 48 hr). Pheromone sources were attached to the tops of the stakes with a No. 4 insect pin and were approximately first ear height. Distance between traps was approximately 25 m. All tests were conducted in cornfields near Brookings, South Dakota, in July and August 1982.

RESULTS AND DISCUSSION

Response by NCR males to the individual stereoisomers is shown in Table 1. Male NCR were attracted only to those traps baited with the 2*R*,8*R* isomer; this is the same configuration preferred by WCR males, and probably the only configuration produced by WCR females (Guss et al., 1983). This result is consistent with earlier work that showed that NCR males are attracted

TABLE 1. RESPONSE BY *D. barberi* MALES TO STEREOISOMERS OF 8-METHYL-2-DECYL PROPANOATE

Isomer (1.0 μg)	Mean No. NCR/trap ± SD[a]
2*R*,8*R*	123.5 ± 48.2 a
2*S*,8*R*	2.0 ± 1.4 b
2*R*,8*S*	5.7 ± 3.6 b
2*S*,8*S*	4.7 ± 3.0 b
Solvent blank	5.7 ± 3.8 b

[a]Four traps for each treatment were deployed for two days. Total beetle counts for each trap for two days were pooled. Means followed by the same letter are not significantly different. (Duncan NMRT, $\alpha = 0.05$, $N = 4$).

to volatiles from WCR females (Guss, 1976). The high degree of stereospecificity shown here and the fact that virgin females of both the NCR and WCR are attractive to males of both species (Bartlet and Chiang, 1977) suggest that the sex pheromone of the NCR is largely, if not exclusively, 8*R*-methyl-2*R*-decyl propanoate. In the absence of direct analysis, however, we acknowledge that other compounds may be involved.

These two species occupy the same habitat at the same time, but there is ample evidence that they have only recently become sympatric, which might account for use of the same sex pheromone (Krysan et al., 1982). In those fields in which the NCR and WCR cohabit, it is common to observe instances of apparent interspecific matings. In the vast majority of those sightings, the putative interloper is the NCR male (V.M. Kirk, personal communication). Since both species apparently use the same sex pheromone, such behavior would not be unexpected. The fact that the NCR male is far more likely to engage in interspecific encounters may reflect a lower response threshold to the pheromone. Guss et al. (1982) found that the response threshold for the NCR to racemic 8-methyl-2-decyl propanoate was approximately 10 times less than that for the WCR.

Notwithstanding frequent observations of interspecific mountings and one report that hybrids were obtained in the laboratory (Hintz and George, 1979), Krysan and Guss (1978) showed that, although hybridization between the NCR and WCR is possible, reproductive barriers exist between the two species such that hybridization in nature is unlikely. Evidence of such barriers included a very low incidence of insemination in interspecific pairings, occurrence only of conspecific insemination in competitive experiments involving males of one species and females of both species, and very low viability of eggs resulting from interspecific inseminations.

When the active isomer, 2*R*,8*R*, was mixed individually with the other three in a 1:1 ratio, inhibition of the response of the NCR to 2*R*,8*R* was observed with both the 2*S*,8*R* and 2*S*,8*S* configurations while no apparent effect was shown with 2*R*,8*S* (Table 2). These results explain our earlier finding that traps baited with relatively high levels (10 μg) of racemic 8-methyl-2-decyl propanoate do not capture significant numbers of NCR males (Guss, et al., 1982).

The relative effect of the two inhibiting isomers is shown in Table 3. As little as 0.1 μg of 2*S*,8*R* in these preparations reduced captures to essentially that of the solvent blank, whereas reduction in captures was not apparent with 2*S*,8*S* until at least 0.5 μg were present. With 2*S*,8*R*, captures significantly less than those of the solvent blank began to appear when the inhibiting isomer was present at levels of 0.5 μg or more. This would suggest that the presence of 2*S*,8*R* in relatively high levels results in actual avoidance rather than simply blocking perception by the NCR of 2*R*,8*R*.

In an earlier study, we concluded that the configuration of the natural

TABLE 2. RESPONSE BY *D. barberi* MALES TO MIXTURES OF STEREOISOMERS OF 8-METHYL-2-DECYL PROPANOATE CONTAINING 2*R*,8*R* IN COMMON

Isomer mixture	Mean No. NCR/trap ± SD[a]
1 μg 2*R*,8*R* + —	175.0 ± 21.6 a
1 μg 2*R*,8*R* + 1 μg 2*R*,8*S*	154.0 ± 44.3 a
1 μg 2*R*,8*R* + 1 μg 2*S*,8*S*	68.7 ± 17.7 b
1 μg 2*R*,8*R* + 1 μg 2*S*,8*R*	7.0 ± 3.5 c
Solvent blank	12.0 ± 3.2 c

[a]Four traps for each treatment were deployed for three days. Total beetle counts for each trap for three days were pooled. Means followed by the same letter are not significantly different. (Duncan NMRT, $\alpha = 0.05$, $N = 4$).

pheromone produced by the WCR was probably 2*R*,8*R*, despite the finding that the male WCR responds to both 2*R*,8*R* and 2*S*,8*R* (Guss et al., 1983). Central to our argument was the fact that males of *D. porracea* Harold respond to racemic 8-methyl-2-decyl propanoate, respond only to 2*S*,8*R* among the resolved stereoisomers, and do not respond at all to unfractioned volatiles from female WCR known to be attractive to WCR males. The other two isomers, 2*R*,8*S* and 2*S*,8*S*, were considered to be unlikely components since neither produced any discernable biological activity when tested with the WCR.

The results in the present study and those found earlier are wholly consistent with the above. Thus, Guss (1976) found that unfractionated volatiles from virgin female WCR were highly attractive to NCR males, and Bartelt

TABLE 3. EFFECTS OF VARYING LEVELS OF 2*S*,8*R* AND 2*S*,8*S* ON RESPONSE BY *D. barberi* MALES TO 8*R*-METHYL-2*R*-DECYL PROPANOATE

	Mean No. NCR males/trap ± SD[a]	
Isomer mixture	2*S*,8*R*	2*S*,8*S*
1 μg 2*R*,8*R*	72.8 ± 15.7 a	131.6 ± 43.6 a
1 μg 2*R*,8*R* + 0.1 μg	30.0 ± 8.5 b	133.8 ± 33.6 a
1 μg 2*R*,8*R* + 0.25 μg	17.4 ± 7.0 cd	113.0 ± 25.1 ab
1 μg 2*R*,8*R* + 0.5 μg	12.0 ± 1.6 de	88.4 ± 27.2 bc
1 μg 2*R*,8*R* + 1.0 μg	6.0 ± 3.1 e	68.6 ± 17.3 cd
1 μg 2*R*,8*R* + 2.5 μg	3.2 ± 2.8 e	51.8 ± 24.6 d
Solvent blank	25.0 ± 2.9 bc	37.8 ± 8.1 d

[a]Five traps for each treatment were deployed for five days. Total beetle counts for each trap for five days were pooled. Means followed by the same letter are not significantly different. (Walter-Duncan K ratio (SAS) = 100, $N = 5$).

and Chiang (1977) observed that females of both species were attractive to males of either species. Our finding in this study that males of the NCR are severely inhibited in their response to 2*S*,8*R* by the presence of low levels of 2*S*,8*R* would tend to corroborate the conclusion that 2*S*,8*R* is not a component of the natural WCR pheromone.

The inhibition of NCR attraction to 2*R*,8*R* by 2*S*,8*R* may have a role to play in reproductive isolation between NCR and *D. longicornis* (Say). These two taxa were considered to be subspecies until Krysan et al. (1983) reexamined the *longicornis* complex and elevated the NCR to species rank. We have recently found that male *D. longicornis* respond only to 2*S*,8*R* among the resolved isomers of 8-methyl-2-decyl propanoate (P.L. Guss and J.L. Krysan, unpublished data).

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